Public Health Reports

Vol. 55 • MARCH 1, 1940 • *No. 9*

A STUDY OF PNEUMOCOCCUS TYPING SERUMS FOR THE PURPOSE OF STANDARDIZING A TEST FOR POTENCY [1]

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Since the identification of the types of pneumococci other than I, II, and III by Cooper and her coworkers (*1*, *2*, *3*, *4*) the problems of the preparation and standardization of pneumococcus typing serums have increased in number and importance. An accurate survey of prevalence and mortality due to the different types and the success of treatment with therapeutic serums is, first of all, dependent upon specific and potent typing serums.

Early in the investigations on specific types it was noted that many serums showed cross reactions; that is, they caused an agglutination or capsular swelling reaction not only with the homologous type pneumococcus but with one or more heterologous types of pneumococci as well. Some cross reactions appeared regularly as a characteristic of certain type strains of pneumococci, for example, types II and V, and types III and VIII (*3*, *4*, *5*, *6*). Other cross reactions were found in the serum of certain individual animals and have not been explained satisfactorily. Cooper used absorption tests for checking cross agglutination reactions during her studies on the "higher" types that had previously made up the old group IV pneumococci. When the Neufeld test came into use as a means of typing it was found that absorption of the serum removed the factor responsible for the Neufeld reaction as well as the agglutinins.

The tests for cross reactions remain a laborious necessity to be carried out on every pneumococcus typing serum, but the removal of a cross reaction can be accomplished once it is found. The techniques for absorption vary, but in general the procedure consists in adding a dense suspension of pneumococci of the heterologous type giving the cross reaction to the serum. After agglutination and capsular swelling of the added pneumococci occur, the bacteria are removed from the serum.

[1] From the Division of Biologics Control, National Institute of Health.

The task of setting a standard for the potency of typing serum proved more difficult. Different laboratories worked out their own methods of standardization and minimum requirements, but they varied one from another.

The attack on the problem was begun in this laboratory by collecting samples of typing serums produced in the commercial laboratories and testing them by different methods. The methods first used were modifications of the regular Neufeld test as described by Bullowa (*6*), and of a potency test described by Clapp, Phillips, and Stahl (*7*). As the work progressed the techniques were changed gradually until a practical standard method of procedure was adopted.

In the early tests a 1-mm. loopful of peritoneal fluid of a mouse, moribund following inoculation with an homologous type of pneumococcus, was placed on a glass cover slip. To this was added a 3-mm. loopful of diluted pneumococcus typing serum and a 3-mm. loopful of Loeffler's methylene blue. After thorough mixing the cover slip was inverted onto a clean flat glass slide. The preparations were incubated at 37° C. for 30 minutes and examined microscopically. The end point taken was the highest dilution of serum producing definite capsular swelling. It was soon observed that, while it was possible for one individual to place approximately the same amount of peritoneal fluid on the cover slip each time and thus obtain about the same titer, unless great care was taken it was easy to use more or less peritoneal fluid and obtain widely differing titers. Moreover, the peritoneal fluid of some infected mice contained more leucocytes and more bacteria than other mice so that even when the same loop was used for transferring the peritoneal fluid the titers often varied.

The next tests were performed with broth cultures of pneumococci substituted for the peritoneal fluid of a mouse. The titers obtained with broth cultures were not always the same. If the growth was exceedingly heavy the titer would be lower than if the growth was light. Also, among the various types there were great differences in the appearance of the swollen capsules on pneumococci grown in different lots of broth, with different enrichment media, and in cultures grown for different lengths of time.

It became apparent that there was a need for defining positive and negative Neufeld reactions for purposes of determining potency. A positive reaction was therefore considered to be one in which a preparation showed approximately 90 percent of the pneumococci to have swollen, glassy capsules of a greenish hue with as distinct and definite outlines as those produced by a known positive undiluted rabbit serum. If less than 90 percent of the pneumococci exhibited swollen capsules or if the swollen capsules had hazy, indefinite outlines the test was regarded as negative.

Some lots of broth supported the growth of pneumococci with better capsules than those grown in other lots. The cause of this difference in lots of broth prepared in the same way was not explained. Satisfactory batches of broth were set aside for the preparation of antigens.

Enrichment of broth with blood or serum increased the amount of growth of the pneumococci but added the disadvantage that more debris appeared in the preparations made from such cultures and the end points were often more difficult to read. On the other hand, the addition of dextrose to the broth was distinctly beneficial for the production of satisfactory antigens. Five-tenths percent of dextrose was used. It was noted, furthermore, that when dextrose broth was heavily inoculated with pneumococci and grown but a short time (not less than 1 hour and never more than 5 hours) larger and more definite capsules were present than when a light inoculation of pneumococci was made and the cultures were incubated for a longer period.

Heavy inoculation was accomplished by growing the pneumococci on dextrose blood agar slants from 8 to 12 hours and washing off the growth with 7 cc. of dextrose broth. For types with large capsules, such as types III and XXVII, it was important to remove the cultures from the incubator as soon as, or a little before, they reached their maximum turbidities, or the swollen capsules appeared to have misshapen or fragmented outlines. For types with small capsules, such as types V and XXIV, this precaution was less important.

For preserving the antigens 1.43 percent of a solution of formaldehyde, U. S. P. (0.1 cc. to 7 cc. of culture) was used throughout all the experiments although it will be shown that this exact concentration of formaldehyde was not important. The pneumococci in some of the antigens thus preserved and stored in the refrigerator remained in suspension and retained their normal capsular swelling properties for 2 weeks or more. In others they settled to the bottom of the tube and showed evidences of degeneration within a short time. As a precaution, no antigen was used after it was 4 days old.

It appeared likely that the Neufeld reaction was a quantitative one, depending upon the amount of capsular material to be swollen as well as the amount of antibody present in the serum. For practical purposes, a simple means of measuring the capsular material in the antigen was to measure the turbidity of the dextrose broth culture, using only fully virulent pneumococci from young cultures that possessed good capsules. Any debris present necessarily lessened the number of pneumococci and reduced the capsular material to be swollen. For this reason, after incubation and the addition of formalin the antigen was placed in the refrigerator from 2 hours to overnight to allow any debris from the blood agar or any other extrane-

ous particles or degenerated pneumococci to settle out. After the period at refrigerator temperature, the supernatant fluid containing the pneumococci was diluted with a solution of peptone, sodium chloride, and formaldehyde [2] to match a turbidity standard of 1,000 parts per million of silica (*8*). This was further diluted to match turbidity standards of 800, 600, 400, 200, 100, and 50 parts per million of silica, respectively. Serum dilutions were made in twofold increments in physiological saline solution buffered to pH 7.6. For the test, 0.1 cc. of an antigen was quickly mixed with 0.1 cc. of a serum dilution. Two loopfuls [3] of the mixture were placed on a cover slip and a small loopful [4] of saturated aqueous methylene blue was mixed with it. The cover slip was inverted on a flat glass slide and the preparation was examined under the microscope at once. A sample of the results is shown in table 1. Roughly, doubling the turbidity of the antigen resulted in lowering the titer of the serum one-half, or, conversely, decreasing the turbidity of the antigen by one-half doubled the titer of the serum. This factor of variation of the pneumococci in an antigen must explain, at least in part, the great differences in the titers that have been assigned to the same serum by different laboratories.

TABLE 1.—*The protocol of a test showing the influence of the turbidity of an antigen upon the titer of a serum*

Type II antipneumococcic rabbit serum dilutions	Type II antigen diluted to match turbidity standards of—						
	50 p.p.m. silica	100 p.p.m. silica	200 p.p.m. silica	400 p.p.m. silica	600 p.p.m. silica	800 p.p.m. silica	1,000 p.p.m. silica
1:4	+	+	+	+	+	+	±
1:8	+	+	+	+	+	±	0
1:16	+	+	+	+	±	0	0
1:32	+	+	+	0	0	0	0
1:64	+	±	0	0	0	0	0
1:128	±	0	0	0	0	0	0
1:256	0	0	0	0	0	0	0
Titer	1:64+	1:32+	1:32	1:16	1:8+	1:4+	<1:4

+ = Positive. Swollen capsules with definite outlines equal in size to those produced by undiluted homologous rabbit serum.
± = Almost positive. The capsules not as wide or the outlines not as definite as those produced by the undiluted control serum.
0= Negative. No swollen capsules or swollen capsules with only hazy, indistinct outlines.

An antigen corresponding to a turbidity standard of 200 parts per million of silica contained enough pneumococci so that when two 3-mm. loopfuls of the serum-antigen mixture were placed on a slide many pneumococci were present in each field yet they were not greatly crowded. It was easy to note at a glance whether all the pneumococci had swollen capsules with definite outlines, whether

[2] The solution contained 1 percent peptone, 0.5 percent sodium chloride and 1.43 percent of a solution of formaldehyde, U. S. P., and was buffered to pH 7.6.
[3] A loop of 26-gage platinum, 3 mm. inside diameter.
[4] A loop of 28-gage platinum, 1 mm. inside diameter.

there were any that showed no swollen capsules, or whether agglutination had occurred. Thus, for the sake of convenience, an antigen corresponding to a turbidity standard of 200 parts per million of silica was chosen as the standard antigen for potency tests.

In order to make each antigen as uniformly equal to the turbidity standard as possible, 2 cc. of the supernatant suspension of pneumococci were removed from the stock antigen and 1 cc. was diluted with physiological saline solution to match the turbidity standard. A quantity of peptone solution equal to the quantity of saline solution required to make the dilution was added to the remaining 1 cc. of stock antigen and was used for the tests. This procedure made it possible to eliminate any error due to matching the turbidity of the pneumococci in the amber-colored peptone solution with the colorless turbidity standards. Table 2 shows that the results of tests carried out on serums with carefully standardized antigens prepared on different days were essentially the same.

TABLE 2.—*Results of testing serums with carefully standardized antigens prepared from homologous types of pneumococci on different days*

Antipneumococcic rabbit serum	Date of test	Titer	Antipneumococcic rabbit serum	Date of test	Titer
Type I, lot A	Oct. 21, 1938	1:35	Type XIII (concentrated), lot A	Dec. 2, 1938	1:180
	Oct. 27, 1938	1:40		Jan. 19, 1939	1:180
	Dec. 23, 1938	1:35	Type XIV (concentrated) lot A	Nov. 15, 1938	1:200
Type III (concentrated), lot A	Nov. 8, 1938	1:50		Nov. 29, 1938	1:180
	Nov. 10, 1938	1:50	Type XVI, lot A	Apr. 28, 1939	1:16
	Dec. 16, 1938	1:50		May 3, 1939	1:16
	Feb. 21, 1939	1:50	Type XIX (concentrated), lot A	Nov. 10, 1938	1:230
Type III (concentrated), lot B	Oct. 24, 1938	1:40		Jan. 12, 1939	1:180
	Oct. 25, 1938	1:35	Type XX (concentrated), lot A	Jan. 16, 1939	1:200
	Nov. 8, 1938	1:40		Jan. 18, 1939	1:180
Type V, lot A	Jan. 31, 1939	1:20	Type XXV, lot A	Apr. 26, 1939	1:4+
	Feb. 14, 1939	1:16		May 2, 1939	1:4+
Type VIII, lot A	July 7, 1939	1:8+	Type XXVIII, lot A	Apr. 28, 1939	1:8
	Aug. 4, 1939	1:8+		May 2, 1939	1:8
Type IX (concentrated), lot A	Jan. 16, 1939	1:140		May 6, 1939	1:8+
	Jan. 17, 1939	1:160			
	Jan. 19, 1939	1:180			

Clinically, pneumococci are typed from sputum whenever possible. For this reason it was of interest to find out whether a viscous material, such as is usually found in sputum from pneumonia patients, interfered in any way with the combination of capsular substance and antibody. Table 3 shows that approximately 5.2 percent of mucin interfered to the extent that the titer of the serum was reduced by one-half. The lower concentrations of mucin did not lower the titer of the serum but the end points were difficult to read because the outlines of many of the capsules were obscure.

That this interference with the Neufeld reaction was not a characteristic of mucin alone was shown when a viscous material composed of a 50 percent aqueous solution of dextrose was used for diluting the

stock antigen or serum, or both. It will be noted that as the concentration of the dextrose was increased the titer of the serum was decreased (table 3a). Unlike the preparations containing mucin, the end points of the preparations containing dextrose were easily read.

TABLE 3.—*Effect of different concentrations of mucin on the titer of a serum*

Dilutions of antipneumococcic rabbit serum type, II, lot B	Control (no mucin)	Mucin approximately 2.2 percent	Mucin approximately 3 percent	Mucin approximately 5.2 percent
	Stock antigen diluted 1:4 with peptone solution, serum diluted with saline solution	Stock antigen diluted 1:4 with 6 percent mucin, serum diluted with saline solution	Stock antigen diluted 1:4 with peptone solution, serum diluted with 6 percent mucin	Stock antigen diluted 1:4 with 6 percent mucin, serum diluted with 6 percent mucin
1:8	+	[1] +	[1] +	[1] +
1:16	±	[1] ±	[1] ±	0
1:32	0	0	0	0
Titer	1:16	[1] 1:16	[1] 1:16	[1] 1:8

[1] End point difficult to read.

TABLE 3a.—*Effect of different concentrations of dextrose on the titer of a serum*

Dilutions of antipneumococcic rabbit serum, type II, lot A	Control (no dextrose)	Dextrose approximately 18.7 percent	Dextrose approximately 25 percent	Dextrose approximately 43.7 percent
	Stock antigen diluted 1:4 with peptone solution, serum diluted with saline solution	Stock antigen diluted 1:4 with 50 percent dextrose solution, serum diluted with saline solution	Stock antigen diluted 1:4 with peptone solution, serum diluted with 50 percent dextrose solution	Stock antigen diluted 1:4 with 50 percent dextrose solution, serum diluted with 50 percent dextrose solution
1:4				+
1:8			+	+
1:16	+	+	+	±
1:32	±	0	0	0
1:64	0	0	0	0
Titer	1:32	1:16	1:16	1:8+

From the results of the tests with mucin and dextrose it seemed reasonable to conclude that in specifying the minimum requirements for the potency of pneumococcus typing serums some allowance should be made for the fact that the pneumococci in sputums are often surrounded by a thick tenacious material, and therefore require a stronger serum than if they were suspended in a liquid such as broth or peptone solution. It did not seem practicable to employ a viscous material for the dilution of the antigen or serum in potency tests, since such a diluent would involve standardization of the preparation and viscosity of the material. If a satisfactory standardization of the material could be accomplished, it would still not correspond to the interfering substances that might be present in sputums, since sputums vary widely, even from the same patient.

Methylene blue being a factor in the Neufeld test, an effort was made to determine what effect different concentrations and amounts added at different stages of the test might have upon the titer of a serum. It was observed that methylene blue could be used with equal success in different amounts, depending upon the concentration, for the regular Neufeld test performed with undiluted rabbit serum. The same amounts of solutions of methylene blue were added on the cover slip to the serum-antigen mixtures in tests for the potency of typing serums. Inasmuch as each serum-antigen mixture was further diluted on the cover slip by the dye solution, tests were carried out to determine the effect of this dilution. First, the methylene blue was added to the saline solution used for making the serum dilutions, making any further dilution of the serum-antigen mixture unnecessary, and, second, the volume of the serum-antigen mixtures in the test tube was increased four times by the addition of saline solution containing methylene blue. Tests were also performed in which methylene blue was added to the antigen before it was mixed with the serum dilution to find out whether the capsules appeared more distinct if the pneumococci were stained before the capsules were swollen rather than afterwards. The results are given in table 4. It will be noted (columns 1, 2, and 3) that the amounts of methylene blue solution that were satisfactory for demonstrating the swollen capsules in the regular Neufeld test served equally well in quantitative capsular swelling tests and (columns 4 and 5) that the added dilution of the serum-antigen mixture by the methylene blue solution had no effect upon the titer of a serum. On the other hand, coloring the pneumococci before the capsules were swollen (column 6) was sometimes a disadvantage. The outlines of the swollen capsules were often more indistinct and the end points were difficult to read.

To determine whether formalin influenced the results of the assay of a serum, a series of experiments was carried out with a portion of a stock antigen to which 1.43 percent of a solution of formaldehyde, U. S. P., was added and with a portion which contained no solution of formaldehyde. The results were the same, as is shown in table 5. It may be stated, however, that some differences might have occurred had not other precautions been taken for preserving the antigens. Antigens were kept in the refrigerator at all times when not in use and in an iced container while being used. They were used for a period not longer than 4 days and only when the pneumococci remained in the broth suspension; antigens in which the pneumococci had settled to the bottom of the tube were discarded.

A series of experiments was also carried out with antigens to which were added 0.25, 0.5, and 1.43 percent, respectively, of a solution of formaldehyde (table 5). No advantage of one concentration over the other was noted. The results were in keeping with those of

TABLE 4.—*Comparison of quantitative Neufeld reactions when different concentrations of methylene blue were added after the serum-antigen mixtures were made and when methylene blue was added to the serum dilutions or antigen before the serum-antigen mixtures were made*

Antipneumococcic rabbit serum dilutions	Methylene blue solutions added to serum-antigen mixture on cover slip			Methylene blue solution added to serum-antigen mixture in test tube to increase the serum dilution 4 times	Methylene blue solution added to saline solution used for making serum dilution	Methylene blue solution added to antigen before serum-antigen mixture was made
	Two 3-mm. loopfuls of serum-antigen mixture + one 1-mm. loopful of saturated aqueous methylene blue	Two 3-mm. loopfuls of serum-antigen mixture + one 3-mm. loopful of 10 percent saturated aqueous methylene blue	Two 3-mm. loopfuls of serum-antigen mixture + one 5-mm. loopful of Loeffler's methylene blue	0.2 cc. of serum-antigen mixture + 0.6 cc. methylene blue solution (0.5 cc. saturated aqueous methylene blue to 9.5 cc. saline)	(1 cc. of saturated aqueous methylene blue to 25 cc. of saline)	Antigen equal to a silica standard of 400 p. p. m. diluted with equal parts of a solution containing 1.5 cc. of saturated aqueous methylene blue in 8.5 cc. of peptone solution
Type I, lot B 1:8	+	+	+			
1:16	+	+	+			
1:32	+	+	+			
1:64	0	0	0			
1:128	0	0	0			
Titer	1:32	1:32	1:32			
Type I, lot C 1:8	+			+		
1:16	+			+		
1:32	±			±		
1:64	0			0		
Titer	1:16+			1:16+		
Type XXVIII, lot B 1:4		+			+	
1:8		0			0	
Titer		1:4			1:4	
Type XXXII, lot A 1:4		+			+	
1:8		+			+	
1:16		+			+	
1:32		+			+	
1:64		0			0	
Titer		1:32			1:32	
Type XIV, concentrated. 1:160						+
1:170	+					+
1:180	+					0
1:190	0					0
1:200	0					0
Titer	1:180					1:170
Type I, lot B 1:8	+	+	+	+	+	+
1:16	+	+	+	+	+	+
1:32	±	±	±	±	±	0
1:64	0	0	0	0	0	0
Titer	1:16+	1:16+	1:16+	1:16+	1:16+	1:16
Type II, lot C 1:8	+	+	+	+	+	+
1:16	+	+	+	+	+	+
1:32	±	±	±	±	±	0
1:64	0	0	0	0	0	0
Titer	1:16+	1:16+	1:16+	1:16+	1:16+	1:16
Type VIII, lot B 1:16	+	+	+	+	+	+
1:32	+	+	+	+	+	+
1:64	0	0	0	0	0	0
Titer	1:32	1:32	1:32	1:32	1:32	1:32
Type XXXII, lot B 1:16	+	+	+	+	+	+
1:32	±	±	±	±	±	0
1:64	0	0	0	0	0	0
Titer	1:16+	1:16+	1:16+	1:16+	1:16+	1:16+

Barnes and Hager (quoted by White (*9*)) who found that the amount of combined formaldehyde was the same irrespective of whether 0.2, 0.3, 0.4, or 0.5 percent of formalin had been added to a culture of pneumococci.

TABLE 5.—*Assay of antipneumococcic serums with antigens containing no formaldehyde and with antigens containing different concentrations of a solution of formaldehyde*

Antipneumococcic rabbit serum dilutions		No formaldehyde	0.25 percent of a solution of formaldehyde U. S. P.	0.5 percent of a solution of formaldehyde U. S. P.	1.43 percent of a solution of formaldehyde U. S. P.
Type I (concentrated) lot E	1:32	+			+
	1:64	+			+
	1:128	+			+
	1:256	0			0
Titer		1:128			1:128
Type IX, lot B	1:32	+			+
	1:64	+			+
	1:128	0			0
Titer		1:64			1:64
Type XXVII, lot A	1:32	+			+
	1:64	+			+
	1:128	0			0
Titer		1:64			1:64
Type III, lot C	1:16				+
	1:32		+	+	+
	1:64		0	0	0
Titer			1:32	1:32	1:32
Type VIII, lot C	1:16		+	+	+
	1:32		±	±	±
	1:64		0	0	0
Titer			1:16+	1:16+	1:16+
Type II, lot A	1:32		+	+	+
	1:64		0	0	0
Titer			1:32	1:32	1:32
Type III, lot D	1:16		+		+
	1:32		+		+
	1:64		0		0
	1:128		0		0
Titer			1:32		1:32

Throughout all the investigations, the peptone solution used for diluting the antigens and the saline solution used for diluting the serums were adjusted to pH 7.6. The final pH of the antigen was in most cases somewhat less than pH 7.6, owing to acids produced by growth of the pneumococci in the dextrose broth medium. Whether or not the variation of pH was a factor of any importance was tested in the following manner: Portions of a stock antigen were adjusted with hydrochloric acid or sodium hydroxide to pH 5.6, 7.6, and 8.0, respectively, and diluted to match a silica turbidity standard of 200 parts per million with peptone solutions of the same pH values. Triplicate serum dilutions were then made with physiological saline solution buffered to pH 5.6, 7.6, and 8.0, and tests were performed with the antigen and serum dilutions of each corresponding pH value (table 6). The pH values tested had no effect upon the quantitative Neufeld reaction under the conditions of the experiments.

That the pH may be a factor in the keeping qualities of antigens was suggested. When antigens adjusted to pH 5.6, 7.6, and 8.0 were observed after they had remained in the refrigerator for a period of

3 weeks, the majority of the pneumococci in the more alkaline suspensions, pH 7.6 and 8.0, had dropped to the bottom of the tube, whereas little sedimentation had occurred in the suspension of pH 5.6. Also, the pneumococci in some of the more alkaline antigens, when tested with homologous type serums, exhibited more swollen capsules with misshapen or ragged outlines than the swollen capsules in the antigens adjusted to pH 5.6. Other factors involved in the keeping qualities of antigens for periods of 2 to 3 weeks, or longer, were not investigated.

TABLE 6.—*Assay of a serum with antigens and saline solution used for making the serum dilutions adjusted to pH 5.6, 7.6, and 8.0, respectively*

Antipneumococcic rabbit serum dilutions		pH 5.6	pH 7.6	pH 8.0
Type II, lot A	1:4	+	+	+
	1:8	+	+	+
	1:16	+	+	+
	1:32	+	+	+
	1:64	0	0	0
	1:128	0	0	0
Titer		1:32	1:32	1:32
Type III (concentrated), lot E	1:32	+	+	+
	1:64	±	±	±
	1:128	0	0	0
Titer		1:32+	1:32+	1:32+

The question of whether enough soluble specific substance was dissolved in the culture medium to cause a variation in the titer of a serum was considered. Stock antigens were prepared as usual and portions were adjusted to pH 5.6, 7.6, and 8.0, respectively, diluted with peptone solutions of the same pH values, and the antigens of pH 5.6 and 8.0 and portions of the antigen of pH 7.6 were centrifugalized. The clear supernatant fluid of each tube was decanted and the sedimented pneumococci were resuspended in the same amount of peptone solution of the respective pH previously used (table 7). With the exception of one test, no differences in the titer of a serum were observed when antigens of pH 5.6, 7.6, and 8.0, centrifugalized and resuspended in peptone solution of the same pH, were used. This exception was with antigens in which many of the pneumococci showed damaged swollen capsules when tested with an homologous type serum and the end points were difficult to read.

There were differences in 4 of 6 tests, however, when antigens which had not been centrifugalized were compared with those which had. In one test some of the pneumococci in the centrifugalized antigen exhibited only slightly swollen capsules or swollen capsules with fragmented outlines when tested with the higher dilutions of the homologous type serum. For this reason, the titers of the serum were lower as judged by the usual method of regarding as positive only preparations in which approximately 90 percent of the pneu-

mococci showed swollen capsules with definite outlines. In 3 tests the titers of the serums tested with antigens which had been centrifugalized and resuspended in peptone solution were higher. It is probable that in these experiments some of the capsular material or pneumococci were lost in removing the supernatant liquid from the centrifugalized antigens.

TABLE 7.—*Comparisons of the titers of serums tested with antigens adjusted to pH 5.6, 7.6, and 8.0, centrifugalized and resuspended in peptone solution of the same pH values and antigens which had not been centrifugalized, adjusted to pH 7.6*

Antipneumococcic rabbit serum dilutions	Antigens not centrifugalized	Antigens centrifugalized and resuspended in peptone solution		
	pH 7.6	pH 5.6	pH 7.6	pH 8.0
Type II, lot A 1:16			+	
1:32	+	+	+	+
1:64	0	±	±	±
1:128	0	0	0	0
Titer	1:32	1:32+	1:32+	1:32+
Type II, lot A 1:32	+		+	+
1:64	0		0	0
Titer	1:32		1:32	1:32
Type III, lot F 1:8	+			+
1:16	+	+	+	±
1:32	+	±	±	0
1:64	0	0	0	0
1:128	0	0	0	
Titer	1:32	1:16+	1:16+	1:8+
Type III, lot F 1:16	+		+	
1:32	+	+	+	+
1:64	0	+	+	+
1:128	0	+	+	+
1:256	0	0	0	0
Titer	1:32	1:128	1:128	1:128
Type V, lot B 1:8	+		+	
1:16	+	+	+	+
1:32	±	±	±	±
1:64	0	0	0	0
1:128	0	0	0	0
Titer	1:16+	1:16+	1:16+	1:16+
Type VIII, lot C 1:16	+	+	+	+
1:32	±	+	+	+
1:64	0	0	0	0
Titer	1:16+	1:32	1:32	1:32

That the titer of a serum was not consistently higher or lower when tested with an antigen which had been centrifugalized and resuspended in peptone solution was shown in two instances with antigens prepared and tested at different times. For both serums, the titers obtained with the antigens prepared on different days which had not been centrifugalized were the same, but in neither test were the titers obtained with the antigens centrifugalized and resuspended in peptone solution the same. It was concluded, therefore, that centrifugalizing and resuspending the pneumococci in an antigen was a time-consuming procedure more prone to introduce an error than to correct one.

Taking into consideration the factors of the Neufeld reaction which had been investigated, a standard test was evolved and adopted for

testing the potency of diagnostic pneumococcus serums of all types received from the different laboratories. The test was as follows:

Materials.—The saline solution used for diluting the antipneumococcus rabbit serum was a solution of 0.85 percent sodium chloride buffered to pH 7.6.

The methylene blue was a saturated aqueous solution.

The antigen was prepared by washing the growth of fully virulent pneumococci from an 8- to 18-hour dextrose blood agar slant with 7 cc. of 0.5 percent dextrose broth that had previously been tested for supporting good growth and capsule production of pneumococci. The broth culture was incubated at 37° C. from 1 to 5 hours (never over 5 hours), depending upon the turbidity. Care was taken, particularly for types with large capsules, that the broth cultures were removed from the incubator as soon as, or a little before, the maximum turbidity was reached. The pneumococci were killed by adding 0.1 cc. of a solution of formaldehyde, U. S. P., to each 7 cc. of culture (1.43 percent). After the addition of the formaldehyde the antigen was stored in the refrigerator for at least 2 hours and usually overnight. Before use, 2 cc. of the supernatant suspension were pipetted off and 1 cc. was diluted with saline solution to match as exactly as possible a turbidity standard containing 200 parts per million of silica. This tube was discarded. To the remaining 1 cc. a quantity of peptone solution[5] was added that was equal to the quantity of saline solution required to match the turbidity standard. This antigen was tested with undiluted rabbit serum of the homologous type and if the pneumococci were found to have swollen, glassy capsules of a greenish hue with definite outlines, the antigen was considered satisfactory for the test. Such swollen capsules were used as standards for judging the capsular swelling of each serum dilution.

The antigen was kept in the refrigerator when not in use and in an iced container while being used. It was not used for longer than 4 days, and was not used after the pneumococci dropped out of suspension or after the capsules of the pneumococci swollen by the homologous type serum appeared to have outlines that were fragmented or hazy and indistinct.

The test.—One-tenth cc. of the serum of half the dilution being tested and 0.1 cc. of the antigen were mixed well in a small test tube. Two loopfuls [6] were placed on a cover slip and a small loopful [7] of saturated aqueous methylene blue was mixed with it. The cover slip was inverted on a flat glass slide and the preparation examined under the microscope after 2 to 4 minutes.

[5] The solution contained 1 percent peptone, 0.5 percent sodium chloride, and 1.43 percent of a solution of formaldehyde, U. S. P., and was buffered to pH 7.6.

[6] A loop of 26-gage platinum, 3 mm. inside diameter.

[7] A loop of 28-gage platinum 1 mm. inside diameter.

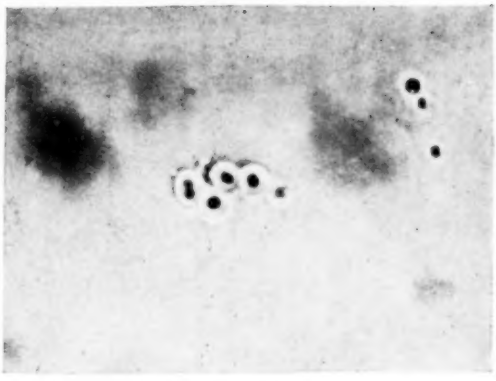

FIGURE 1.—Positive Neufeld reaction. The capsules are swollen by undiluted rabbit serum of the homologous type. Note the wide capsules with definite outlines.

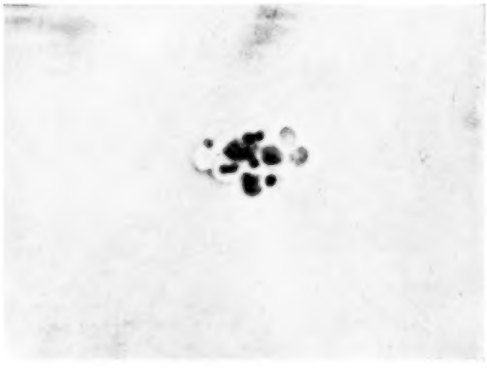

FIGURE 2.—Plus-minus Neufeld reaction. The capsules are not as wide or the outlines are not as definite as those produced by the undiluted control serum.

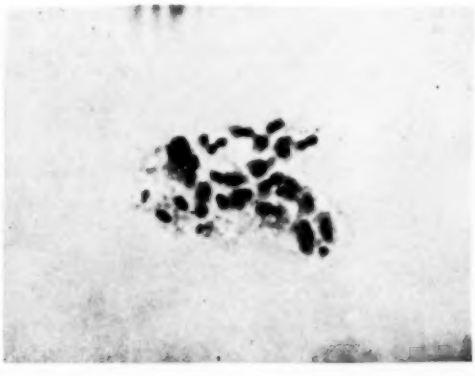

FIGURE 3.—Negative Neufeld reaction. Some of the pneumococci have slightly swollen capsules but the outlines are hazy and indistinct.

The only preparations regarded as positive were those in which at least 90 percent of the pneumococci exhibited swollen capsules as definite and distinct as those produced by a known positive undiluted rabbit serum of the homologous type. If the number of pneumococci with definitely swollen capsules was less than 90 percent, or if the outlines of the capsules were hazy and indistinct, the serum dilution was read as negative. Border-line preparations in which the outlines of the swollen capsules failed to be quite as distinct or in which the capsules failed to be quite as wide as those produced by the undiluted control serum were read plus-minus (figs. 1, 2, and 3).

Particular attention was necessary in reading the results of tests with pneumococci having large capsules, for example, types III and XXVII. Several fields were examined to make certain that each serum dilution caused at least 90 percent of the pneumococci to have swollen capsules with definite outlines. Sometimes in high dilutions of serum a few of the pneumococci would show large definitely swollen capsules while the remainder would show none.

Types of pneumococci showing narrow swollen capsules when tested with homologous type serum, such as types V, XIV, and XXIV, were found to swell somewhat more slowly than pneumococci with wide capsules. A time limit of 2 to 4 minutes was, however, sufficient for these types.

The antipneumococcus typing serums assayed by this method revealed that the titers ranged from 0 to, in a few serums, 1:128. In other words, some of the undiluted serums produced only slightly swollen capsules, with outlines too indistinct to be regarded as positive by the adopted definition of a positive Neufeld reaction. With the exception of the type III monovalent serums, titers of 1:4 to 1:8 were most frequently encountered for all types. Type III serums were uniformly low in potency.

After completion of tests of all the typing serums collected and collection of data concerning the use of different typing serums in clinical laboratories, an arbitrary titer for the minimum requirement for all types except type III was placed at 1:16 for monovalent serums and 1:8 for typing serum mixtures. The potency for monovalent type III serum and for type III in serum mixtures was placed at 1:8 and 1:4, respectively.

As the Neufeld test is carried out in most clinical laboratories more typing serum than sputum or mouse peritoneal fluid is used. Taking this fact into consideration, the arbitrary minimum titers chosen for typing serums allow a small margin of safety for obtaining a positive reaction when sputum is unusually thick and tenacious, when large numbers of pneumococci of the homologous type are present, and for deterioration due to such little investigated factors as aging, temperature, or light. It may be mentioned that the titers chosen for the

minimum requirements were higher than the titers of many of the typing serums available in 1937 and 1938.

DISCUSSION

The experiments demonstrate that the most important factors in the test of potency of pneumococcus typing serums are the careful preparation and standardization of the antigen and uniformity in reading the results. In other words, the Neufeld reaction represents a quantitative combination of the capsular component of the pneumococcus and the antibody in the serum. Some measure, direct or indirect, of the capsular component must be made if the amount of antibody is to be estimated. In general, reducing the turbidity of the antigen by one-half (or the capsular material by one-half) doubles the titer of a serum.

This quantitative relationship of capsular substance and antibody is in keeping with the technique of typing pneumococci from sputum or other pathological materials considered as satisfactory by Cooper and Walter (*4*); they used 4 to 40 times as much serum as sputum or other pathological material. It is again suggested in the warning by White (*10*) that in dealing with sputum in which many type III organisms are present it is sometimes necessary to dilute the sputum with salt solution before any swelling of the pneumococcus capsule is evident.

In reading the results attention has been drawn to the importance of a swollen capsule with a definite outline, rather than simply a swollen capsule. The definiteness of the outline is dependent principally upon the potency of the serum. The width of a swollen capsule is usually characteristic of the type of the pneumococcus, although the virulence of the strain, the age of the culture, and the medium in which it is grown affect the capsular width. It is because of the differences in width of the swollen capsules that an undiluted homologous-type rabbit serum should be used as a control for judging whether each test serum dilution is negative or positive.

The fact that two viscous materials, mucin and a concentrated solution of dextrose, interfered with the Neufeld reaction was of interest because of the practice of typing pneumococci from sputum, mouse peritoneal fluid, or other pathological materials that are often thick and viscous. While it is not practicable to use a viscous material as a diluent for either the antigen or serum dilutions in tests for the potency of pneumococcus typing serums it was a point that was considered when the arbitrary minimum standards were chosen.

The experiments in which different concentrations of methylene blue were added to the serum-antigen mixtures show that methylene

blue added after the serum-antigen mixture is made has no essential effect except to dye the pneumococci and bring out the swollen capsules. That the further dilution of the serum due to the addition of the methylene blue solution to the serum-antigen mixture does not influence the results tends to simplify the standard test. Methylene blue can be added to the saline solution used for making the serum dilutions, but no advantage is derived from it. The end points are sometimes lower and more difficult to read when methylene blue is added to the antigen before the serum-antigen mixtures are made.

The experiments demonstrating that the concentration of formalin in the antigen or the hydrogen ion concentration of the test materials can be varied over a considerable range without affecting the titer of a serum make the proposed test of potency easier to duplicate. It is to be borne in mind, however, that the antigens are not used for a period longer than 4 days.

CONCLUSIONS

1. A quantitative Neufeld test for determining the potency of pneumococcus typing serum is described.

2. Minimum requirements for monovalent pneumococcus typing serums and for group typing serum mixtures are proposed.

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YELLOW FEVER

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Yellow fever is a disease with which the general public is not very familiar at present. In the past it was a terrible scourge in sections of the United States, causing many thousands of deaths. It was so dreaded that when an epidemic occurred great numbers of people would desert the stricken area, fleeing until stopped, often by men with shotguns, at the borders of districts still free from the disease.

According to historical records, yellow fever made its first appearance in North America in the year 1668, when there were severe epidemics in New York and Philadelphia. During the early history of the United States yellow fever epidemics were recorded from New Hampshire to Florida, as far west as Texas, and up the Mississippi River as far as St. Louis. Between the years 1668 and 1821 there were no less than 20 different epidemics in Philadelphia, 15 in New York, 8 in Boston, and 7 in Baltimore.

An account of early epidemics in this country will be found in Dr. LaRoche's book on yellow fever published in Philadelphia in 1855. In speaking of the epidemic of 1793 in Philadelphia, when there were over 4,000 deaths, the author describes the terror which seized the population when the existence of yellow fever in the city was officially announced. People either fled to the country or shut themselves up in their homes. Friends avoided meeting in the streets, and handshaking fell into disuse. Unemployment was universal, and business came to a standstill. The death rate was so high that burying parties worked day and night disposing of the dead. During the 10 years following this epidemic there were 4 more outbreaks in Philadelphia claiming an additional 10,000 lives.

While the northern cities were visited by epidemics of yellow fever at relatively infrequent intervals, in some of the southern towns the infection was present almost continuously. In Charleston, S. C., deaths from yellow fever occurred practically every year in the nineteenth century. There were frequent and violent epidemics in Galveston, New Orleans, and a number of other Gulf ports. From New Orleans the infection was frequently carried inland along the Mississippi River. The last epidemic of yellow fever in the United States occurred in New Orleans in 1905, at which time there were cases also in Pensacola, Fla.

Yellow fever is still widespread in the interior of South America and in West Africa. Some authorities are inclined to believe that it was originally brought to the New World from West Africa on slave ships.

THE COURSE OF YELLOW FEVER IN MAN

Yellow fever derives its name from the jaundiced or yellow color of the skin, mucous membranes, and sclerae, which commonly develops about the third or fourth day of illness. The onset is usually very sudden, and patients frequently remember the exact hour when they were taken ill. The disease begins with a severe headache, backache, and fever. From the very beginning the patient feels extremely sick and prostrated. There is frequent vomiting. After the first 2 or 3 days the vomit often contains altered blood, hence the common name "black vomit." Yellow fever is a disease of rather short duration, and at the end of 1 week most victims are either dead or on their way to recovery. In fatal cases death usually occurs between the fifth and eighth day of illness. If the patient survives until the seventh day, his chances for recovery are generally good. There are exceptions to this statement, of course. In fulminating cases death may occur as early as the end of the third day, and in less severe cases as late as the ninth or tenth day. Before death the patient usually falls into a coma which may last from 12 to 24 hours. The death rate varies in different epidemics, but generally it is high in comparison with other infectious diseases.

The diagnosis of yellow fever during the first few days of illness is very difficult and almost impossible without laboratory aid. The most important early symptoms for diagnosis are high fever with slow pulse rate, leucopenia, and albuminuria; and later, jaundice, bleeding gums, and vomiting of altered blood.

There is no specific treatment for yellow fever. The best that can be done is to keep the patient quiet in bed and on light liquid diet, as in other severe illnesses.

One attack confers lifelong immunity. In places where the disease has occurred year after year, practically all the adult population has had the infection and thus is immune, so children and newcomers are the chief victims. In some persons the attack is so mild that it can be diagnosed only after recovery by testing the patient's blood for immunity.

ETIOLOGY AND MODE OF TRANSMISSION

The infectious nature of yellow fever was known long ago; but our knowledge regarding the causative agent and its mode of transmission from one person to another is quite recent, dating from the close of the Spanish-American War, when the United States Army Commission under Major Walter Reed demonstrated in Cuba that yellow fever is transmitted by a mosquito formerly called the stegomyia but now known under the scientific name of *Aëdes aegypti* and popularly designated as the aegypti. The Commission showed also that the causative

agent was present in the patient's blood during the first 3 days of illness, and that it was small enough to pass through filters which held back ordinary bacteria. In addition, the Commission demonstrated that the patient's sputum, feces, urine, and vomitus were noninfectious, and that the disease could not be contracted by contact or from contaminated objects.

Major (later General) W. C. Gorgas, who was the Chief Sanitary Officer of the United States Army of Occupation in Cuba at the time, made quick use of this knowledge. Cuba, and especially the city of Havana, had long been a notorious hotbed of yellow fever, and there were many deaths among the American soldiers stationed there. All ordinary sanitary measures had met with complete failure. Early in 1901 Gorgas introduced vigorous antimosquito measures in Havana, and as a result yellow fever disappeared as if by magic. Three years later Gorgas, by applying similar measures in the Panama Canal Zone, made possible the building of the Panama Canal.

Although the brilliant work of Major Reed and General Gorgas provided measures for preventing the spread of yellow fever, information on many important points regarding this disease was not forthcoming until much later. The chief reason for this delay was the lack of a susceptible animal in which the infection could be studied experimentally. It was only in 1927, or 27 years after Major Reed's work, that the members of the West African Yellow Fever Commission of the Rockefeller Foundation first succeeded in transmitting yellow fever to rhesus monkeys, and most of our knowledge regarding the causative agent of yellow fever has been obtained since that date. Later other animals, especially white mice and European hedgehogs, were also found to be susceptible.

We know now that the causative agent of yellow fever belongs to that class of infectious agents known as filterable viruses. It readily passes through bacteria-tight porcelain filters and is not visible even with the most powerful microscope; in fact, it is one of the smallest viruses known. It is a strict parasite in the sense that it will not multiply in the absence of living tissue cells. Outside a living host it dies out very rapidly unless special precautions are taken. It can be maintained, however, in tissue cultures as long as the tissue cells continue to live. By frequently replacing old tissues by fresh ones in such cultures, it has been possible to cultivate yellow fever virus outside a living host continuously for a number of years. Investigators noticed that in the course of prolonged cultivation under such artificial conditions the virus lost much of its virulence, and when injected into susceptible animals, such as monkeys, it produced immunity but not disease. At present such a modified virus is used for human vaccination against yellow fever on a large scale.

Before an effective vaccine was developed by the staff of the International Health Division of the Rockefeller Foundation in 1931, a number of scientists engaged in studying yellow fever in various laboratories during the 4 years between 1927 and 1931 contracted the infection accidentally, and several paid with their lives. Among the latter were Drs. Adrian Stokes, Hideyo Noguchi, William A. Young, Paul Lewis, and Theodore Hayne, whose untimely deaths were a great loss to medical science. Vaccination was introduced in May 1931, and since then there has not been a single accidental infection among the investigators.

The mechanism by which the mosquito transmits yellow fever is as follows: The virus is present in the circulating blood of an infected person, not only during the first 3 or 4 days of illness, but also at least 6 hours, and probably even longer, before the onset of fever, when the patient is still feeling perfectly well. An aegypti mosquito biting a person in this stage probably ingests several thousand infective doses of virus with its normal blood meal. The mosquito then becomes a virus carrier for the rest of its life. After the infective meal about 12 days elapse before the virus reaches the salivary glands of the mosquito. But after it has reached there, the mosquito will inject some of the virus into each subject it bites. The virus causes no harm to the mosquito itself. Although the duration of its normal life in nature is not definitely known, a mosquito infected with yellow fever virus has been kept alive in the laboratory for over 200 days.

After a susceptible person has been bitten by an infected mosquito, there follows an incubation period during which the virus multiplies in the person's body. During this period, which is usually from 3 to 5 days, but in rare instances may be as long as 10, he is feeling perfectly normal and is entirely unaware that he is carrying dangerous infection within himself. Mosquitoes that bite him toward the end of this period or during the first 3 or 4 days of illness will in turn become virus carriers, and after about 12 days they will be ready to infect new victims. In this manner the infection can persist in a community indefinitely if there are enough mosquitoes and a sufficient number of nonimmune human beings.

EPIDEMIOLOGY OF YELLOW FEVER

Yellow fever is best known as the aegypti-borne disease of cities, but it exists also in tropical forests in the absence of this mosquito and is then called "jungle yellow fever." This jungle yellow fever is primarily a disease of lower animals and is only accidentally transmitted to man. Its permanent home is in tropical forests of the interior of South America, and it is probable that the disease has a similar epidemiology in parts of Africa. Exactly what animals

serve as its host in these jungles is not yet known. We do know, however, that persons visiting jungles known to be infected contract the disease, probably from a bite of some bloodsucking insect. The yellow fever contracted in the jungle differs in no essentials from that occurring in aegypti-infected cities. Moreover, a person infected in the jungle entering a community where there is heavy aegypti breeding may serve as a source of infection for the mosquitoes and initiate an epidemic of yellow fever of the classical urban type.

There are other mosquitoes besides aegypti capable of transmitting yellow fever. Most of these are found only in tropical countries, especially Africa and South America. They have not acquired domestic habits like aegypti and generally do not breed in and around human habitations. These wild jungle mosquitoes seldom come into contact with man and therefore they play an insignificant role in the spread of yellow fever in the cities, although it would seem likely that they may serve as a source of infection contracted in the jungle.

The chief yellow fever vector, the aegypti, is, on the other hand, distinctly domestic in its habits. It breeds almost exclusively in and around houses. Its favorite breeding places are artificial water containers such as cisterns, tanks, buckets, roof gutters, and empty cans and bottles which have been filled with water during rain. In houses it often breeds in flower vases, icebox drainage pans, and other vessels in which water is left standing for some length of time. It has practically never been found breeding in swamps, rivers, lakes, or other places where malaria mosquitoes usually breed. In cities with a modern pipe-borne water supply and a sanitary sewage disposal system the number of available breeding places is limited and relatively easy to control. However, in regions where yellow fever is still prevalent most towns lack such modern facilities.

The yellow fever mosquito breeds only in a warm climate, and this explains why yellow fever epidemics have been more frequent in the southern States than in the northern. On the other hand, as shown by the occurrence of epidemics in Philadelphia, New York, and Boston, it can also breed during the summer months in the northern latitudes if introduced from the Tropics and furnished with suitable breeding places. In the Tropics it breeds in abundance and constitutes one of the major pests.

In the days of the old sailing ships drinking water on these vessels was stored in open wooden tanks which afforded excellent breeding places for aegypti mosquitoes. These vessels were the chief carriers of yellow fever infection from one port to another. This also explains why yellow fever epidemics occurred most frequently in seaports and cities on important maritime trade routes. In modern steamships the water supply is carried in closed steel tanks where mosquito breeding is impossible. But the faster modern ships and airplanes

afford easier transportation not only for infected persons but also for adult mosquitoes.

Three elements are essential for the outbreak of yellow fever in an urban community. They are an infected person, mosquitoes, and a nonimmune population. If the aegypti-infested towns are within a reasonable traveling distance from areas where jungle yellow fever is prevalent, there is always the threat that sooner or later some person will contract the infection in the jungle and unknowingly bring it into the town. Towns which previously were several days away from a jungle by mule are now only a few hours away by motor bus. The danger of a town becoming infected from the jungle increases as modern transportation facilities improve.

PREVENTION AND CONTROL OF YELLOW FEVER

Inasmuch as it is not yet known what animals and insects play a part in maintaining yellow fever in the jungle, there is very little we can do to prevent the spread of the infection in the forest itself. Certain measures can be taken, however, to prevent human beings from contracting the disease in the forest and bringing it to communities where there are conditions favorable for its spread. Most effective of these measures is vaccination against yellow fever. A person actively immunized will not be able to harbor the virus even if he should come into contact with it in the jungle. Because of this it is strongly urged that in regions where there is reason to suspect the presence of jungle yellow fever all those likely to come into contact with it in the forest be vaccinated. This applies especially to labor forces engaged in road building, forest clearing for new industrial or agricultural developments, or cutting firewood.

Although it is important to protect individuals exposed to infection in the jungle, it is of far greater importance to protect all large urban population centers and render them noninfectible, on the chance that the infection might accidentally be brought in. The most effective way to accomplish this is to rid the community of the transmitting agent—the aegypti mosquito. Without means of transmission yellow fever cannot spread. In order to devise effective measures for the eradication of the aegypti it is necessary to know something of its life and habits.

As mentioned above, the aegypti breeds only in and around houses and is found in artificial containers almost exclusively. Only females bite, and blood is essential for the development of their ova. They lay their eggs near the surface of water on the side of any container they find, but in general prefer clean water and practically never breed in sump pits or sewer drains. If the eggs remain moist, they will hatch out in about 24 to 48 hours. If, however, they become dry, hatching will be retarded, but the eggs remain viable and will

hatch if submerged in water even months later. The larvae continue to grow for 7 to 9 days until they reach the pupal stage. From the pupae the adult mosquitoes emerge about 2 days later. Thus in a very warm, tropical climate at least 10 days are required before the mosquito reaches the adult stage from the egg, and during this period it lives only in water. In cooler climates the time required to reach maturity is several days longer.

It is the aquatic stage in the cycle of development of the mosquito that is most susceptible to control measures. If all water containers are completely emptied at least once a week, the cycle is broken, and this simple procedure is sufficient to prevent mosquitoes from reproducing. As a matter of fact, this is the procedure generally employed in the control of urban yellow fever and, if carried out carefully, it usually gives excellent results.

Departments of public health are ordinarily responsible for the enforcement of antimosquito measures aimed at yellow fever. Methods have been brought to a high degree of perfection in Brazilian cities. Specially trained sanitary inspectors visit all houses at weekly intervals. Householders are instructed to keep their premises clean and to dispose of all unnecessary water containers. Water storage tanks are mosquitoproofed with wire screen. Courtyards and surroundings of houses are kept clean, and all discarded receptacles, such as empty tin cans, broken bottles, and the like, which are likely to hold rain water, are disposed of. Householders are requested to cooperate in every way with the sanitary inspectors in their effort to eradicate mosquito breeding. Those who fail to do so are sometimes fined or even more severely punished. In large cities it is at times exceedingly difficult to locate all breeding places; therefore, special skill and previous experience on the part of the inspector is essential. If antimosquito measures as outlined above are successfully introduced in an aegypti-infested community, the adult mosquitoes, as a rule, disappear within a month. If adult aegypti continue to be found in some particular houses, this indicates that there is an undiscovered breeding focus somewhere in the vicinity. Some breeding places which cannot be entirely eliminated are rendered harmless by periodical oiling, as the film of oil will kill the mosquito larvae. Large tanks are sometimes stocked with special varieties of small fish that feed on mosquito larvae.

When a case of yellow fever is discovered in an aegypti-infested town, the patient is isolated in mosquitoproof quarters to prevent mosquitoes from biting him and becoming virus carriers. As an added precaution, the house in which he was taken ill is usually fumigated for the purpose of destroying adult mosquitoes that may already have bitten him and become infected. Strict antilarval measures are imme-

diately introduced throughout the community, and mosquito breeding is thus brought under control.

During recent years rigid antimosquito measures have been enforced in many of the seaports of South and Central America and the West Indies. As a result, no serious epidemics of urban yellow fever have occurred since 1928 and 1929, when there was an outbreak in Rio de Janeiro. Although there have been many cases of jungle yellow fever in the interior of South America every year, strict antimosquito measures have prevented the spread of the infection to coastal cities. However, as long as the immense tropical hinterland of the southern continent continues to serve as a potential source of infection, the danger of its spread will persist. During periods of war or earthquake the rigid systematic control measures are likely to break down, and as soon as this happens seaports again become infested with aegypti mosquitoes and yellow fever epidemics reappear.

This situation constitutes a grave danger to all infectible countries, including the warmer regions of the United States. That our southern cities are still infectible is shown by the recent epidemics of dengue fever, a disease transmitted from one person to another by the same mosquito that transmits yellow fever, *Aëdes aegypti*. Until a few years ago travel between South America and the United States was possible only by steamship. Because of great distances and the slowness of the ship, the voyage usually took many days or even weeks. If a ship happened to carry infection, it was usually detected during the voyage. Appropriate quarantine measures were taken when a United States port was reached, and the spread of infection was prevented. Ships coming from known infected ports were invariably quarantined. The effectiveness of these measures is suggested by the fact that there has been no yellow fever in the United States since 1905.

With the development of air transportation the situation has changed greatly. Thousands of persons now travel by air every year between the United States and South America. It is thus possible for a person bitten by an infected mosquito in South America to arrive in the United States by airplane and to travel around in this country for 2 or 3 days before actually becoming ill with yellow fever acquired in distant Tropics. In the first days of his illness, and before it is possible to diagnose his infection definitely, he is capable of infecting large numbers of aegypti mosquitoes, provided these insects are present in his community. He may thus serve as a source for an epidemic outbreak of yellow fever. Moreover, there is a possibility that infected adult mosquitoes may be trapped in the airplane and be carried mechanically from one country to another.

The responsibility for preventing the introduction of yellow fever into the United States rests with the officers of the United States

Public Health Service who are constantly on guard at the various quarantine stations. When a vessel arrives from a port known or suspected to be infected with yellow fever, all on board are examined and the vessel is inspected to determine whether it is free from mosquitoes. Airplanes are likewise examined and often sprayed with insecticides to kill mosquitoes. Persons arriving by water or air from localities known to be infected, who are not in possession of vaccination certificates, are held under surveillance for the remainder of the yellow fever incubation period, counting from the time of the last possible exposure to infection. The flying personnel of the air lines operating between the United States and the various South American countries are vaccinated against yellow fever.

As a further measure of prevention, all those who expect to travel in regions where yellow fever is prevalent are urged to be vaccinated against this disease. Vaccination is now applied on a large scale in places where there is danger of infection. In 1938 more than one million persons were vaccinated in Brazil alone. In the United States a supply of vaccine is kept ready for use by the quarantine officers at the South American air-line terminals in Miami, Fla., and Brownsville, Tex.

Unfortunately, yellow fever vaccine cannot be made available for general distribution. It was mentioned above that the vaccine consists of yellow fever virus rendered nonvirulent by prolonged cultivation in tissue cultures. But in order to produce immunity, the virus in the vaccine must be in a living or active state, since dead or inactive virus will not immunize. Yellow fever virus is very labile, and outside an animal or insect host it rapidly becomes inactivated. It can be rendered much more stable, however, if the virus-containing material or vaccine is thoroughly desiccated in the frozen state and kept in the icebox. Even when stored at low temperature, however, the vaccine becomes inactivated at a rate which is often unpredictable, and, therefore, it has been necessary to test its activity in susceptible animals every time it is used for human immunization. Testing of vaccine activity in animals requires special laboratory facilities which practicing physicians and others who occasionally might wish to use the vaccine for human immunization generally do not have at their disposal. For this reason the availability of yellow fever vaccine in the United States has been limited to certain centers where its application can be controlled scientifically. For the present the vaccine is available under certain conditions and without charge at the Laboratories of the International Health Division of the Rockefeller Foundation in New York, where it was first developed. It is also being given in Rio de Janeiro, Bogotá, London, and Paris. In recent years, missionary, commercial, and governmental organizations, whose employees are stationed in regions of tropical Africa or South America

where there is danger of yellow fever infection, have made extensive use of these facilities.

NOTES ON A VARIATION IN THE EGGS OF *ANOPHELES PUNCTIPENNIS* SAY

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During the routine examination of batches of eggs laid by captive *Anopheles punctipennis* adults some unusual eggs were encountered. These eggs were so strikingly different from the ordinary or usual type of *punctipennis* eggs that they were at once noticed. In reporting them as a possible seasonal variation it is hoped that other investigators may extend and confirm these observations.

In the study of anopheline eggs the terminology used by Howard, Dyar, and Knab (1912, as corrected) and by Christophers and Barraud (1931), and other writers is followed. The *dorsal surface* of the egg is the upper surface, as the egg floats normally on the water. The *ventral surface* is the underside covered almost, if not entirely, by the water and this surface is clothed with a delicate membrane, the *exochorion*, which gives the ventral surface its silvery appearance. The *anterior end* is the larger and more blunt end. The *frill*, a delicately lobate, ribbon-like membrane around the borders of the exposed portion of the dorsal surface of the egg, is sometimes difficult to see unless lighting is properly applied. The *floats* are specialized portions of the exochorion and occur one on each side of the egg, and are composed of several compartments or cells. The *endochorion* (or *chorion* of some authors) lies beneath the exochorion.

Eggs were obtained by allowing individual wild-caught female mosquitoes to oviposit directly on the surface of water in small crystallizing dishes (50 mm. diameter) beneath "Sport" type lantern chimneys. Only one mosquito was placed in each chimney. To prevent eggs from becoming stranded on the sides of the dish, a small paraffin-coated cork ring was floated on the water.

For study, eggs were placed on wet filter paper on an ordinary microscope slide without cover glass, where they remained motionless. Both transmitted and reflected light played an important part in proper illumination. Eggs to be preserved for future use were placed on filter paper wet with 4-percent formalin and enclosed in tightly stoppered glass vials. It was found that 2-percent formalin did not entirely prevent hatching. Handling of individual eggs was most successfully accomplished by means of straight, fine-pointed dissecting forceps, using one arm of the forceps only and never closing them.

Photographs were made with a 10–X apochromatic objective, 7.5–X ocular, and a photomicrographic camera whose film holder was

10 inches from the eye point of the microscope. For photography, a "hanging drop" slide, without cover glass, was found most satisfactory, the eggs being floated on a drop of water or formalin. A surface illuminator attached to the microscope gave most satisfactory results as a light source.

In the literature there apparently has been no reference to a seasonal variation in *Anopheles punctipennis* eggs, such as has been noted in *A. walkeri* (figs. 1 and 2) by Matheson and Hurlbut (1937), nor any variation from the usual, such as the egg described here. The description of the egg of *punctipennis* given by Howard, Dyar, and Knab (1917) does not mention the most obvious feature of these possibly seasonal eggs (or "unusual" type, as the writer has designated them for the purposes of this note), namely, the continuation of the exochorion dorsally until it almost completely covers the "deck" or dorsal surface of the egg (fig. 3). The picture of the egg of this species given by the above authors (1912, fig. 694) is distinctly that of the "usual" type of *A. punctipennis* egg and corresponds with their text description previously cited. Herms and Freeborn (1920) describe, and Herms and Frost (1932) describe and illustrate the egg of *punctipennis* as encountered in California. While their description and figures do not correspond exactly with the "usual" type of egg found in Georgia, they more nearly resemble the "usual" type egg than they do the "unusual" type. There are several differences, such as total length, frill, float length, and the number of float compartments, between the "usual" type found in Georgia and the California eggs. These differences are being studied in further detail.

The first female *punctipennis* which laid the "unusual" type eggs was captured at Magnolia Springs, Jenkins County, Ga., on January 21, 1938. Another larger lot of females captured on February 3 also laid "unusual" eggs. Among these females captured on February 3 was one which laid the "usual" type of eggs. This female was indistinguishable in appearance from the others of the lot. Subsequent to February 3 several other adult females captured at Magnolia Springs laid "unusual" eggs. The observations could not be carried on throughout the year. However, it was noted that as summer approached the numbers of "unusual" type eggs obtained from females decreased and that there seemed to be an intergradation between the "unusual" and the "usual" type of eggs.

The character of the exochorion covering the "deck" or dorsal surface of the "unusual" *punctipennis* egg is distinctly different from that in the winter egg of *Anopheles walkeri*. In the latter species, reticulations are observed in this membrane as it covers the ventral surface of the egg and are plainly visible on the dorsal surface under the microscope (fig. 2). In *punctipennis* eggs, while these hexagonal

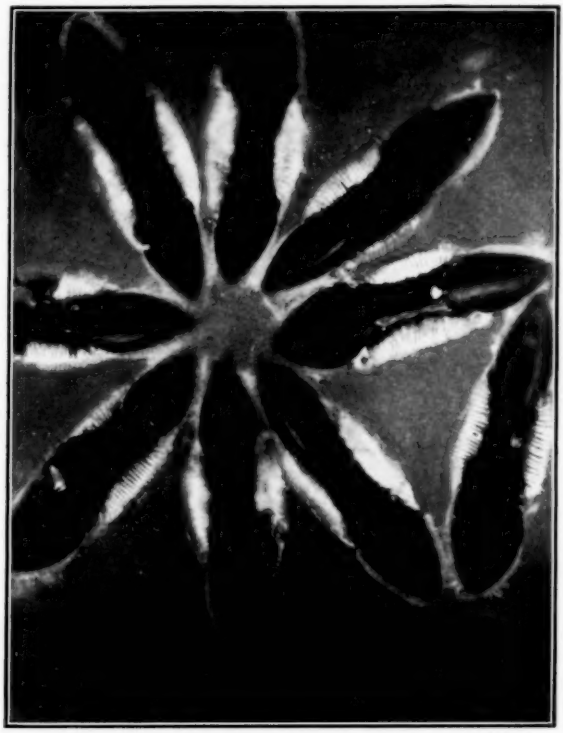

FIGURE 1.—*Anopheles walkeri* Theobald. Summer eggs, dorsal aspect.

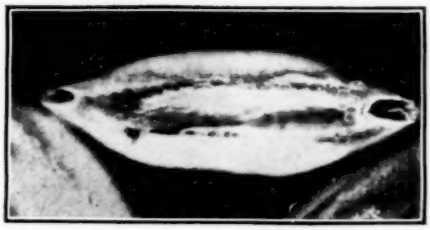

FIGURE 2.—*Anopheles walkeri* Theobald. Winter egg, dorsal aspect.

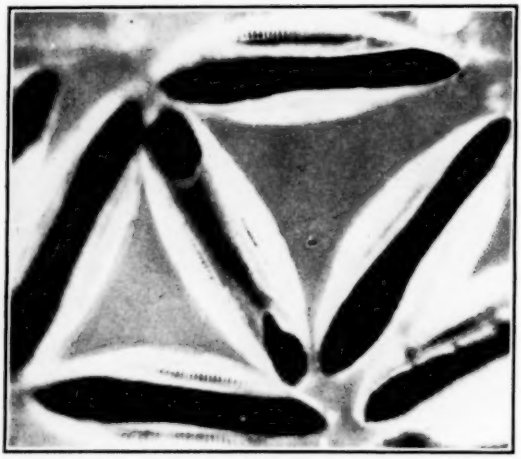

FIGURE 3.—*Anopheles punctipennis* Say. "Unusual" type egg (center) and "usual" type eggs, dorsal aspect. Females have not been observed to oviposit both types of eggs in the same batch.

FIGURE 4.—*Anopheles punctipennis* Say. "Unusual" type egg showing extent and nonreticulated character of exochorion covering dorsal surface of the egg. The two perforations near each end of the membrane were not artificially produced, but are not constant characters. Dorsal aspect.

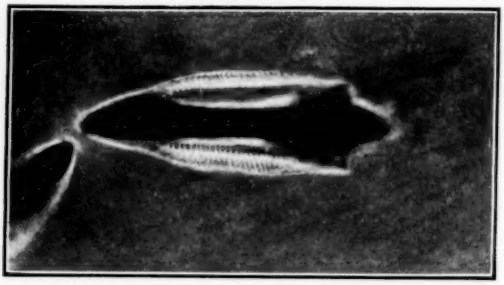

FIGURE 5.—*Anopheles punctipennis* Say. "Usual" type immed ately after hatching. Dorsal aspect.

reticulations are easily discernible on the ventral surface, they are barely visible on the dorsal aspect (fig. 4). Then, too, in the case of "unusual" *punctipennis* eggs the extent of the "deck" covered by the exochorion is not so great. Measurements of the two types of *punctipennis* eggs and counts of the cells of the float chambers have failed to show any significant differences, in contrast to *Anopheles walkeri*, in which the winter egg is larger than the summer egg (Matheson and Hurlbut, 1937), and apparently contains more cells in the floats. The micropylar structures of the two types of *punctipennis* eggs are apparently similar.

All of the *punctipennis* females which laid eggs during this study were the typical, clearly marked, large-spotted type. Howard, Dyar, and Knab (1917) state that the striking variation in the extent and clearness of the pale scaling on the wings found among *punctipennis* "does not even represent a local race, but merely an extreme in the ordinary line of variation." Smaller, darker specimens found in abundance at Magnolia Springs did not lay eggs in the laboratory.

SUMMARY AND CONCLUSIONS

1. Wild-caught *Anopheles punctipennis* females laid "unusual" type eggs in January and February 1938. The female mosquitoes which laid these "unusual" eggs were morphologically indistinguishable from those that had been laying "usual" type eggs during other months of the year.

2. Further studies are necessary before it can be definitely stated that the "unusual" type egg here described is a seasonal variation.

ACKNOWLEDGMENT

In connection with the preparation of this paper the writer desires to acknowledge with deep appreciation the assistance and advice of Senior Surgeon T. H. D. Griffitts, director of the Henry R. Carter Memorial Laboratory at Savannah, Ga., and the members of his staff.

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(*5*) Ibid., vol. 2 (1912), pl. 147, figs. 693–694.

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THE 1940 CENSUS OF POPULATION

The sixteenth decennial census of the United States will be made in April of this year, in accordance with Constitutional provisions. The first United States census was made in 1790 by the United States marshals, and it required a year and a half to complete it, although, according to the final report to Congress, the population of the country was somewhat under 4 million. The 1940 census, covering a population estimated at 132,000,000 and including many new items of information, is expected to be completed in a month. The work will be done by 120,000 enumerators, directed by 528 district supervisors, under the direction of 105 area managers.

The general questions on population will include the name, sex, color or race, age, place of birth, residence, citizenship, education, household data, and household relationships. For persons 14 years of age and over, there are questions relating to employment status and social security. Questions regarding marriage and children will be asked of all women who are or have been married. One in 20 persons will be asked certain questions in order to secure a statistical sample for information on subjects frequently asked the Census Bureau.

Accurate population data for cities, States, and the country as a whole will be especially welcomed by health officers, vital statisticians, and others interested in health and social welfare, for use in the computation of rates; and the age distribution will serve to check the accuracy of theoretical formulae regarding a stabilized population and be useful in other ways.

The census-taking is an enormous task and deserves the full and honest cooperation of all persons in order to make the data as nearly complete and accurate as possible. It will not be a heavy burden on the individual, as many of the answers to the questions can be recorded directly by the census taker without interrogation. The Bureau of the Census states that the average person is not likely to have to answer more than half of the 33 general population questions.

COURT DECISION ON PUBLIC HEALTH

Examination for venereal disease before marriage.—(Illinois Appellate Court, First District, Third Division; *Boysen* v. *Boysen*, 23 N.E.2d 231; decided October 25, 1939.) One provision of the laws of Illinois relating to marriage was as follows:

> All persons desiring to marry shall within 15 days prior to the issuance of a license to marry, be examined by any duly licensed physician as to the existence or nonexistence in such person of any venereal disease, and it shall be unlawful for the county clerk of any court to issue a license to marry to any person who

fails to present for filing with such county clerk a certificate setting forth that such person is free from venereal disease * * *.

Another statutory provision on marriage read:

That if any person residing and intending to continue to reside in this State and who is disabled or prohibited from contracting marriage under the laws of this State shall go into another State or country and there contract a marriage prohibited and declared void by the laws of this State, such marriage shall be null and void for all purposes in this State with the same effect as though such prohibited marriage had been entered into in this State.

A marriage was contracted by residents of Illinois in another State, the parties then returning to Illinois. In a suit for the annulment of the marriage the plaintiff contended that the marriage was void because contracted outside of Illinois to evade the statutory provision regarding examination for venereal disease. In passing upon the matter the appellate court quoted from a prior case, a portion of the quotation being as follows:

In Illinois, "the general rule is that unless the statute expressly declares a marriage contracted without the necessary consent of the parents, or other requirements of the statute, to be a nullity, such statutes will be construed to be directory, only, in this respect, so that the marriage will be held valid although disobedience of the statute may entail penalties on the licensing or officiating authorities." * * *

Neither the statutes of Illinois nor Missouri declare that marriages under a certain age without the parents' consent are void. The provisions fixing the age of consent, requiring the consent of the parents and imposing penalties on the clerk for issuing a license and on officers for celebrating a marriage in violation of the provisions of the statute, are only directory and not prohibitive. * * *

Proceeding, the court used this language:

This court in passing upon the question involved in that case said as to a marriage contracted in a foreign State which does not comply with the requirements of the statute of this State, where the marriage is not declared null and void, that, by this particular statute, the direction of the clerk to issue a license is directory only and not prohibitive. This applies to the facts as we find them in the record in this case, for the venereal disease act does not declare that a marriage celebrated without complying with that statute shall be null and void.

A provision of the statute relating to marriage and the existence or nonexistence in such person of any venereal disease is that it shall be unlawful for the county clerk of any court to issue a license to marry to any person who fails to present for filing a certificate such as is required by the act, and is the only prohibitive clause in the act. * * *

Therefore since the laws of Illinois did not declare void a marriage entered into without compliance with the above-quoted statutory provision as to examination for and freedom from venereal disease, the appellate court rejected the plaintiff's contention that the marriage in the instant case was void.

The order of the lower court, which was adverse to the plaintiff, was affirmed.

DEATHS DURING WEEK ENDED FEBRUARY 10, 1940

[From the Weekly Health Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended Feb. 10, 1940	Corresponding week, 1939
Data from 88 large cities of the United States:		
Total deaths	10,049	9,668
Average for 3 prior years	9,718	
Total deaths, first 6 weeks of year	58,190	55,505
Deaths under 1 year of age	541	574
Average for 3 prior years	582	
Deaths under 1 year of age, first 6 weeks of year	3,307	3,264
Data from industrial insurance companies:		
Policies in force	66,294,279	68,102,528
Number of death claims	13,689	14,277
Death claims per 1,000 policies in force, annual rate	10.8	10.9
Death claims per 1,000 policies, first 6 weeks of year, annual rate	10.5	10.2

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease withou knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED FEBRUARY 24, 1940

Summary

The number of influenza cases reported by the State health officers dropped from 16,548 for the preceding week to 13,950 for the week ended February 24. The preponderance of cases is still being reported from the South Atlantic and South Central States. For the current week, however, all but 3 of the 9 geographic areas registered decreases. The two South Central groups reported considerable decreases, while the South Atlantic States reported an increase, almost entirely accounted for by the rise in the number of cases in West Virginia from 954 to 1,733. All other States in this group reported a decrease, except South Carolina and Virginia, where conditions remained about the same as in the preceding week.

The rise of influenza above the 5-year median began during the week of October 14, 1939, and the numbers of cases reported weekly have remained above the median since that date. The incidence by weeks since the first of the year is as follows:

January				February			
6	13	20	27	3	10	17	24
9,630	12,516	12,568	13,242	17,641	16,583	16,548	13,950

The current incidence of the other 8 communicable diseases included in the weekly telegraphic reports remained below the median expectancy, with the exception of poliomyelitis, which increased from 27 to 30 cases, as compared with the preceding week, while the 5-year median expectancy is 18. Wisconsin reported 5 cases, the highest number for any State.

Telegraphic morbidity reports from State health officers for the week ended February 24, 1940, and comparison with corresponding week of 1939 and 5-year median

In these tables a zero indicates a definite report, while leaders imply that, although none were reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39
	Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939	
NEW ENG.												
Maine	0	4	1		25	5	214	14	44	0	0	0
New Hampshire	0	0	0				49	0	23	0	0	0
Vermont	0	0	0				5	2	4	0	0	0
Massachusetts	4	4	4				292	1,041	400	1	1	2
Rhode Island	0	0	0				111	15	32	0	2	2
Connecticut	0	4	3	2	29	12	185	464	464	0	0	1
MID. ATL.												
New York	25	31	31	[1] 44	[1] 101	[1] 45	319	1,625	1,625	1	5	8
New Jersey	8	10	12	42	44	24	87	25	574	1	0	1
Pennsylvania	25	41	45				98	113	616	11	5	6
E. NO. CEN.												
Ohio	22	17	36	32		53	11	41	108	0	1	8
Indiana	13	13	20	66	1,085	71	5	8	11	0	0	2
Illinois	29	23	31	61	1,478	64	37	19	36	2	2	7
Michigan [2]	7	22	15		255	4	228	447	447	0	0	2
Wisconsin	1	3	3	183	346	134	312	1,065	1,065	0	1	1
W. NO. CEN.												
Minnesota	0	4	4	7	21	1	291	1,246	168	2	0	0
Iowa	4	5	2	42	291	14	158	159	66	1	0	2
Missouri	10	8	23	42		393	3	9	25	0	2	3
North Dakota	3	1	1	23	64	10	3	141	3	0	1	0
South Dakota	0	4	1	1	6	4	2	260	1	0	0	0
Nebraska	1	4	6				29	82	40	2	0	1
Kansas	10	7	9	78	77	22	417	20	20	3	0	0
SO. ATL.												
Delaware	0	2	0				1	0	21	0	0	0
Maryland [2]	0	4	5	107	209	69	4	1,153	136	0	1	7
Dist. of Col	5	7	10	8	25	7	2	19	11	0	2	2
Virginia	18	17	17	2,430	1,604		27	223	269	4	2	6
West Virginia [2]	8	10	10	1,733	36	131	13	49	49	1	3	3
North Carolina [2]	10	22	23	64	230	216	124	1,430	765	0	1	3
South Carolina	5	4	4	1,182	592	604	16	34	34	1	0	2
Georgia [2]	8	5	8	385	110	356	197	232	0	2	1	1
Florida [2]	6	5	7	38		35	65	138	40	0	0	1
E. SO. CEN.												
Kentucky	10	9	9	115	405	405	44	42	243	2	3	9
Tennessee	6	7	12	307	83	246	133	34	38	1	0	8
Alabama [2]	11	13	18	699	180	1,189	44	258	258	1	3	3
Mississippi [2]	9	4	6							0	3	2
W. SO. CEN.												
Arkansas	7	13	11	997	182	182	3	68	60	1	0	2
Louisiana [2]	6	23	15	110	9	24	18	136	70	0	2	2
Oklahoma	8	5	7	487	193	227	7	177	50	0	1	1
Texas [2]	44	35	38	3,448	737	751	414	122	267	1	0	7
MOUNTAIN												
Montana	1	2	2	8	200	132	33	444	30	0	0	0
Idaho	1	1	1	1	12	7	186	74	44	0	0	0
Wyoming	2	1	0				22	67	4	0	0	0
Colorado	8	8	8	35	121		32	95	95	0	1	1
New Mexico	1	0	3	19	3	6	1	21	23	0	0	0
Arizona	3	1	2	291	94	101	16	21	23	1	3	1
Utah	0	0	0	19	44		273	155	26	0	1	0
PACIFIC												
Washington	1	4	4	56		1	529	271	130	0	2	1
Oregon	6	2	1	37	34	143	351	28	28	0	0	0
California	23	32	34	705	59	158	408	3,047	601	3	2	5
Total	369	441	506	13,904	8,987	8,987	5,819	15,134	15,134	42	51	100
8 weeks	3,394	4,483	5,197	112,632	36,759	38,450	37,660	90,202	90,202	307	437	833

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended February 24, 1940, and comparison with corresponding week of 1939 and 5-year median—Continued.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever		
	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39
	Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939	
NEW ENG.												
Maine	0	0	0	13	38	22	0	0	0	0	1	1
New Hampshire	0	0	0	1	1	16	0	0	0	0	0	0
Vermont	0	0	0	13	5	16	0	0	0	0	0	0
Massachusetts	1	0	0	105	233	241	0	0	0	1	1	1
Rhode Island	0	0	0	20	14	17	0	0	0	0	0	0
Connecticut	0	0	0	101	100	88	0	0	0	5	0	0
MID. ATL.												
New York	0	0	0	731	721	793	0	0	0	0	5	5
New Jersey	1	0	0	367	187	174	0	0	0	0	1	1
Pennsylvania	3	0	0	597	408	511	0	0	0	5	4	4
E. NO. CEN.												
Ohio	1	0	0	240	537	493	0	31	7	5	3	3
Indiana	1	0	0	186	217	223	0	136	8	4	0	1
Illinois	1	1	1	656	491	706	6	11	11	5	3	4
Michigan [3]	0	1	0	272	585	585	0	47	3	0	8	3
Wisconsin	5	0	0	174	338	349	6	5	7	4	0	1
W. NO. CEN.												
Minnesota	0	1	0	106	118	169	6	9	9	0	0	0
Iowa	1	0	0	102	159	178	9	34	34	7	1	1
Missouri	0	2	0	87	0	215	12	4	5	0	6	1
North Dakota	0	0	0	50	27	46	1	6	10	1	1	0
South Dakota	0	0	0	22	15	17	0	1	5	0	0	0
Nebraska	0	0	0	21	47	94	0	8	8	0	0	0
Kansas	2	0	0	82	132	209	0	9	9	2	0	0
SO. ATL.												
Delaware	0	0	0	13	0	6	0	0	0	0	0	0
Maryland [3]	0	0	0	43	53	62	0	0	0	0	0	1
Dist. of Col	0	0	0	25	20	20	0	0	0	0	1	1
Virginia	2	2	0	50	33	35	0	0	0	0	2	2
West Virginia [3]	2	0	0	56	51	51	0	0	0	2	5	4
North Carolina [3]	1	0	0	36	56	33	0	0	0	0	4	4
South Carolina	0	0	0	2	5	5	0	0	0	3	2	2
Georgia [3]	1	1	0	19	2	13	1	0	0	2	2	2
Florida [3]	0	1	1	4	10	6	0	0	0	2	1	1
E. SO. CEN.												
Kentucky	0	1	0	84	74	74	0	7	0	4	3	3
Tennessee	0	0	0	86	39	39	0	3	0	1	2	2
Alabama [3]	0	3	2	23	10	12	0	6	1	2	2	2
Mississippi [3]	2	0	0	10	7	12	0	1	1	1	1	1
W. SO. CEN.												
Arkansas	0	1	0	14	17	11	0	5	5	2	0	1
Louisiana [3]	0	0	0	12	16	14	0	0	0	7	46	7
Oklahoma	0	0	0	12	49	31	1	22	6	0	0	0
Texas [3]	3	1	1	53	87	87	4	25	2	5	6	11
MOUNTAIN												
Montana	0	0	0	33	56	47	0	3	11	0	1	1
Idaho	0	0	0	14	9	19	0	4	4	0	0	0
Wyoming	0	0	0	6	13	13	0	1	3	0	0	0
Colorado	0	0	0	81	33	73	17	17	5	0	0	0
New Mexico	0	1	0	13	11	24	1	1	0	1	0	0
Arizona	0	0	0	4	15	16	0	20	0	1	0	0
Utah	0	1	0	26	37	49	0	0	0	0	0	0

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended February 24, 1940, and comparison with corresponding week of 1939 and 5-year median—Continued.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever		
	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39	Week ended		Median, 1935–39
	Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939	
PACIFIC												
Washington	0	1	0	66	53	57	1	5	23	0	1	1
Oregon	0	0	0	24	45	58	0	4	4	2	0	0
California	3	0	1	156	256	242	0	26	9	4	2	4
Total	30	18	18	4,911	5,430	6,901	65	451	283	78	115	113
8 weeks	260	133	174	35,765	42,750	50,571	573	3,201	2,364	621	891	891

Division and State	Whooping cough		Division and State	Whooping cough	
	Week ended			Week ended	
	Feb. 24, 1940	Feb. 25, 1939		Feb. 24, 1940	Feb. 25, 1939
NEW ENG.			SO. ATL.—continued		
Maine	49	11	North Carolina [3]	65	266
New Hampshire	6	1	South Carolina	14	75
Vermont	23	19	Georgia [3]	23	54
Massachusetts	124	225	Florida [3]	8	25
Rhode Island	12	85			
Connecticut	27	65	E. SO. CEN.		
MID. ATL.			Kentucky	47	23
New York	354	568	Tennessee	32	14
New Jersey	55	440	Alabama [3]	19	45
Pennsylvania	267	365	Mississippi [3]		
E. NO. CEN.			W. SO. CEN.		
Ohio	67	213	Arkansas	10	8
Indiana	24	26	Louisiana [3]	30	12
Illinois	105	278	Oklahoma	1	2
Michigan [2]	100	225	Texas [3]	111	64
Wisconsin	97	318	MOUNTAIN		
W. NO. CEN.			Montana	4	15
Minnesota	19	42	Idaho	44	2
Iowa	9	24	Wyoming	6	12
Missouri	11	32	Colorado	3	44
North Dakota	7	10	New Mexico	23	20
South Dakota	0	0	Arizona	23	36
Nebraska	12	8	Utah	100	22
Kansas	39	32	PACIFIC		
SO. ATL.			Washington	26	37
Delaware	4	10	Oregon	36	12
Maryland [2]	153	20	California	198	116
Dist. of Col	24	17	Total	2,508	4,025
Virginia	54	65			
West Virginia [2]	43	22	8 weeks	22,093	34,185

[1] New York City only.
[2] Period ended earlier than Saturday.
[3] Typhus fever, week ended February 24, 1940, 25 cases as follows: North Carolina, 1; Georgia, 4; Florida, 1; Alabama, 5; Louisiana, 3; Texas, 11.

CASES OF VENEREAL DISEASES REPORTED FOR DECEMBER 1939

These reports are published monthly for the information of health officers in order to furnish current data as to the prevalence of the venereal diseases. The figures are taken from reports received from State and city health officers. They are preliminary and are therefore subject to correction. It is hoped that the publication of these reports will stimulate more complete reporting of these diseases.

Reports from States

	Syphilis		Gonorrhea	
	Cases reported during month	Monthly case rates per 10,000 population	Cases reported during month	Monthly case rates per 10,000 population
Alabama [1]				
Arizona	221	5.29	102	2.44
Arkansas	891	4.29	180	.87
California	1,579	2.53	1,378	2.20
Colorado	97	.90	61	.57
Connecticut	181	1.03	123	.70
Delaware	203	7.72	25	.95
Dist. of Columbia	659	10.36	325	5.11
Florida	2,413	14.29	204	1.20
Georgia	1,404	4.51	71	.23
Idaho	34	.68	10	.20
Illinois	1,976	2.50	1,090	1.38
Indiana	490	1.40	142	.41
Iowa	277	1.08	122	.48
Kansas	244	1.31	129	.69
Kentucky	615	2.08	223	.75
Louisiana	483	2.25	97	.45
Maine	39	.45	32	.37
Maryland	795	4.72	265	1.57
Massachusetts	433	.98	335	.76
Michigan	709	1.45	359	.74
Minnesota	224	.84	177	.66
Mississippi	1,808	8.86	2,418	11.85
Missouri	694	1.73	193	.48
Montana	47	.86	28	.51
Nebraska	110	.81	61	.45
Nevada	30	2.94	20	1.96
New Hampshire	16	.31	5	.10
New Jersey	851	1.95	228	.52
New Mexico	135	3.20	60	1.42
New York	3,360	2.59	1,448	1.11
North Carolina	1,871	5.30	187	.53
North Dakota	28	.39	42	.59
Ohio	805	1.19	287	.42
Oklahoma	377	1.47	245	.95
Oregon	115	1.11	102	.98
Pennsylvania	1,072	1.05	111	.11
Rhode Island	69	1.01	25	.37
South Carolina	883	4.69	224	1.18
South Dakota	31	.45	27	.39
Tennessee	1,332	4.56	330	1.13
Texas	3,386	5.43	650	1.04
Utah	41	.79	26	.50
Vermont [1]				
Virginia	1,748	6.37	270	.98
Washington	159	.95	202	1.21
West Virginia	262	1.38	104	.55
Wisconsin	40	.14	74	.25
Wyoming	15	.63	7	.30
Total	29,463	2.62	12,298	1.01

Reports from cities of 200,000 population or over [2]

Akron, Ohio	27	.98	23	.84
Atlanta, Ga	313	10.42	68	2.26
Baltimore, Md	469	5.61	177	2.12
Birmingham, Ala	276	9.38	52	1.77
Boston, Mass	158	1.99	115	1.45
Buffalo, N. Y	74	1.23	48	.80
Chicago, Ill	1,235	3.37	742	2.02
Cincinnati, Ohio	105	2.22	68	1.44
Cleveland, Ohio	370	3.92	80	.85
Columbus, Ohio	102	3.25	15	.48
Dallas, Tex	205	6.74	96	3.16

See footnotes at end of table.

Reports from cities of 200,000 population or over—Continued

	Syphilis		Gonorrhea	
	Cases reported during month	Monthly case rates per 10,000 population	Cases reported during month	Monthly case rates per 10,000 population
Denver, Colo	67	2.22	44	1.46
Houston, Tex	185	5.16	120	3.35
Jersey City, N. J	36	1.11	10	.31
Kansas City, Mo	116	2.69	43	1.00
Los Angeles, Calif	836	5.50	583	3.83
Louisville, Ky	175	5.16	58	1.71
Memphis, Tenn	386	13.22	48	1.64
Milwaukee, Wis	2	.03	10	.16
Minneapolis, Minn	55	1.10	58	1.16
Newark, N. J	121	2.66	200	4.40
New York, N. Y	2,318	3.09	951	1.27
Oakland, Calif	60	1.92	68	2.17
Omaha, Nebr	36	1.61	28	1.25
Philadelphia, Pa	484	2.41	[1]	[1]
Portland, Oreg	75	2.34	78	2.43
Rochester, N. Y	24	.70	52	1.52
St. Louis, Mo	64	.76	68	.81
St. Paul, Minn	37	1.29	20	.70
San Antonio, Tex	459	17.55	52	1.99
San Francisco, Calif	183	2.66	193	2.80
Seattle, Wash	84	2.17	98	2.53
Syracuse, N. Y	65	2.88	11	.49
Washington, D. C	659	10.36	325	5.11

[1] No report for current month.
[2] No reports received from Dayton, Detroit, Indianapolis, New Orleans, Pittsburgh, Providence, or Toledo.

WEEKLY REPORTS FROM CITIES

City reports for week ended February 10, 1940

This table summarizes the reports received weekly from a selected list of 140 cities for the purpose of showing a cross section of the current urban incidence of the communicable diseases listed in the table.

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Data for 90 cities:											
5-year average	183	1,222	147	4,682	965	2,004	36	387	18	1,138	------
Current week [1]	97	1,089	119	1,210	760	1,430	5	340	14	749	------
Maine:											
Portland	0	------	0	34	2	1	0	0	0	14	22
New Hampshire:											
Concord	0	------	0	0	3	0	0	0	0	0	11
Manchester	0	------	1	0	1	0	0	0	0	0	14
Nashua	0	------	0	27	0	0	0	0	0	0	7
Vermont:											
Barre	0	------	------	0	------	0	0	------	0	0	------
Burlington	0	------	0	0	0	0	0	0	0	0	10
Rutland	0	------	0	0	0	0	0	0	0	0	5
Massachusetts:											
Boston	1	------	2	18	7	46	0	4	0	46	235
Fall River	0	------	0	23	1	0	0	1	0	23	30
Springfield	0	------	0	1	3	5	0	0	0	3	42
Worcester	0	------	0	2	15	4	0	0	0	3	73
Rhode Island:											
Pawtucket	1	------	0	8	0	3	0	0	0	0	22
Providence	0	------	0	91	7	8	0	2	0	8	72
Connecticut:											
Bridgeport	0	------	0	2	2	2	0	2	0	0	41
Hartford	0	------	0	0	2	5	0	0	1	8	42
New Haven	0	------	1	0	0	5	0	0	0	6	61
New York:											
Buffalo	0	------	0	3	6	4	0	7	0	6	116
New York	17	36	------	40	------	377	0	------	3	80	1,568
Rochester	0	1	1	5	3	7	0	1	0	6	65
Syracuse	0	------	0	0	5	7	0	1	0	[2]12	56
New Jersey:											
Camden	2	2	2	0	3	4	0	0	0	0	47
Newark	0	4	0	14	6	25	0	3	0	25	125
Trenton	0	1	2	0	10	5	0	4	0	2	58

[1] Figures for Little Rock and for deaths in New York estimated; reports not received.

City reports for week ended February 10, 1940—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Pennsylvania:											
Philadelphia	3	41	8	4	44	53	0	28	0	0	687
Pittsburgh	2	18	9	3	23	37	0	10	0	10	257
Reading	0	------	1	1	4	0	0	0	0	2	24
Scranton	1	------	------	1	------	4	0	------	0	1	------
Ohio:											
Cincinnati	3	1	5	0	11	21	0	6	0	16	156
Cleveland	0	106	3	7	7	34	0	11	1	34	215
Columbus	0	1	1	2	6	9	0	4	0	4	114
Toledo	0	3	1	4	3	10	0	1	0	4	82
Indiana:											
Anderson	2	------	0	0	5	3	0	0	0	8	14
Fort Wayne	0	------	0	0	3	2	0	1	0	0	26
Indianapolis	6	------	1	1	13	23	2	4	1	16	132
Muncie	1	------	0	0	4	6	0	0	0	0	14
South Bend	0	------	1	0	2	4	0	0	0	0	21
Terre Haute	0	------	3	0	5	0	0	1	0	0	38
Illinois:											
Alton	0	------	0	0	3	1	0	1	0	0	11
Chicago	10	34	6	10	58	333	0	31	0	37	779
Elgin	0	------	0	1	2	1	0	0	0	2	14
Moline	0	------	1	0	1	4	0	0	0	0	16
Springfield	0	------	1	0	5	5	0	0	0	0	30
Michigan:											
Detroit	3	1	1	6	18	53	0	12	1	34	274
Flint	1	------	0	0	6	11	0	0	0	15	31
Grand Rapids	0	------	0	4	4	15	0	0	0	4	37
Wisconsin:											
Kenosha	0	------	0	1	0	2	0	0	0	0	4
Milwaukee	0	------	0	1	6	20	0	8	0	5	106
Racine	0	------	0	2	1	2	0	0	0	2	14
Superior	0	------	0	7	0	1	0	0	0	0	12
Minnesota:											
Duluth	0	------	0	300	0	2	0	1	0	0	32
Minneapolis	3	------	0	1	7	23	0	1	0	5	141
St. Paul	0	2	2	1	8	11	0	1	0	11	80
Iowa:											
Cedar Rapids	0	------	------	4	------	2	0	------	0	0	------
Davenport	0	------	------	0	------	3	0	------	0	0	------
Des Moines	0	------	0	3	0	12	0	0	0	1	40
Sioux City	0	------	------	0	------	2	0	------	0	0	------
Waterloo	2	------	------	0	------	5	0	------	0	0	------
Missouri:											
Kansas City	1	9	1	1	19	18	0	6	0	0	110
St. Joseph	0	------	0	0	5	1	0	0	0	0	31
St. Louis	5	32	5	3	30	31	2	13	0	7	252
North Dakota:											
Fargo	0	------	0	0	2	0	0	0	0	0	8
Grand Forks	0	------	------	0	------	0	0	------	0	2	------
Minot	0	------	0	0	0	1	0	0	0	0	4
South Dakota:											
Aberdeen	0	------	------	1	------	2	0	------	0	0	------
Sioux Falls	0	------	0	0	0	8	0	0	0	0	8
Nebraska:											
Lincoln	1	------	------	1	------	1	0	------	0	1	------
Omaha	0	------	0	0	6	8	0	5	0	0	51
Kansas:											
Lawrence	0	------	0	0	0	0	0	0	0	0	10
Topeka	0	1	1	0	4	2	0	0	0	1	18
Wichita	1	2	0	233	6	3	0	1	0	0	35
Delaware:											
Wilmington	0	------	0	0	3	1	0	2	0	8	35
Maryland:											
Baltimore	2	77	2	1	27	24	0	12	0	142	306
Cumberland	0	------	0	0	5	0	0	0	0	0	26
Frederick	0	------	0	0	1	3	0	0	0	0	------
Dist. of Col.:											
Washington	0	19	5	0	29	21	0	16	1	15	226
Virginia:											
Lynchburg	1	------	0	0	3	0	0	0	0	3	19
Norfolk	1	159	0	0	5	2	0	1	0	5	30
Richmond	0	------	2	0	7	3	0	1	0	0	56
Roanoke	0	------	0	1	1	3	0	0	0	2	18
West Virginia:											
Charleston	1	2	0	0	1	0	0	0	0	0	18
Huntington	0	------	------	0	------	2	0	------	0	0	------
Wheeling	0	------	1	0	2	2	0	0	1	1	17

City reports for week ended February 10, 1940—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
North Carolina:											
Gastonia	0	----	----	0	----	0	0	----	0	0	----
Raleigh	0	----	0	0	1	1	0	0	0	0	7
Wilmington	0	----	2	0	2	0	0	0	0	0	21
Winston-Salem	0	2	0	0	1	8	0	0	0	0	10
South Carolina:											
Charleston	0	226	1	0	5	0	0	1	1	0	25
Florence	0	----	0	0	0	0	0	0	0	0	3
Greenville	0	----	0	0	1	0	0	0	0	1	11
Georgia:											
Atlanta	0	69	3	16	15	8	0	5	0	3	110
Brunswick	0	----	0	0	0	0	0	0	0	0	5
Savannah	0	64	2	0	6	1	0	2	0	0	38
Florida:											
Miami	0	13	0	0	7	1	0	2	1	0	60
Tampa	2	7	3	17	8	1	0	0	0	1	48
Kentucky:											
Ashland	0	----	0	0	1	0	0	0	0	2	6
Covington	0	----	0	1	2	2	0	0	0	0	18
Lexington	1	----	0	0	1	3	0	2	0	6	17
Louisville	0	26	0	3	12	34	0	5	0	30	95
Tennessee:											
Knoxville	0	16	3	0	3	16	0	1	0	0	35
Memphis	0	31	6	10	12	29	1	4	0	6	118
Nashville	0	----	3	13	5	2	0	3	0	0	66
Alabama:											
Birmingham	0	27	2	0	9	2	0	3	0	0	94
Mobile	0	11	3	0	4	2	0	1	0	0	35
Montgomery	0	4	----	7	----	0	0	----	0	0	----
Arkansas:											
Fort Smith	1	133	----	0	----	0	0	----	0	0	----
Little Rock	----	----	----	----	----	----	----	----	----	----	----
Louisiana:											
Lake Charles	1	----	0	0	1	1	0	1	0	1	4
New Orleans	9	41	6	1	39	9	0	14	1	4	238
Shreveport	0	----	2	0	22	0	0	0	0	0	60
Oklahoma:											
Oklahoma City	1	52	0	0	6	1	0	3	0	0	50
Tulsa	3	----	----	0	----	11	0	----	0	6	----
Texas:											
Dallas	0	11	3	2	8	3	0	2	0	10	73
Fort Worth	3	----	0	1	4	4	0	0	0	8	34
Galveston	1	----	0	1	4	1	0	0	0	0	22
Houston	6	2	2	3	14	3	0	2	1	4	94
San Antonio	1	48	2	59	18	1	0	7	0	3	107
Montana:											
Billings	1	----	0	0	1	0	0	0	0	0	13
Great Falls	0	----	0	0	1	2	0	0	0	0	14
Helena	0	----	0	0	0	0	0	0	0	0	3
Missoula	0	----	0	0	0	0	0	0	0	1	6
Idaho:											
Boise	0	----	0	1	2	0	0	0	0	0	10
Colorado:											
Colorado Springs	0	----	0	0	2	1	0	0	0	0	18
Denver	3	----	1	2	10	9	0	2	0	0	106
Pueblo	2	----	0	1	0	5	0	0	0	0	11
New Mexico:											
Albuquerque	0	----	0	0	2	2	0	3	0	7	11
Utah:											
Salt Lake City	0	----	1	39	1	9	0	0	0	59	29
Washington:											
Seattle	1	----	1	136	6	8	0	5	0	3	123
Spokane	1	1	1	1	3	7	0	0	0	10	34
Tacoma	0	----	0	51	2	4	0	0	0	0	37
Oregon:											
Portland	0	8	1	58	10	9	0	3	0	9	112
Salem	0	2	----	32	----	0	0	----	0	0	----
California:											
Los Angeles	3	152	4	10	16	24	0	18	1	16	322
Sacramento	0	1	1	1	4	0	0	0	1	0	34
San Francisco	4	4	0	1	9	4	0	10	0	13	188

City reports for week ended February 10, 1940—Continued

State and city	Meningococcus meningitis		Poliomyelitis cases	State and city	Meningococcus meningitis		Poliomyelitis cases
	Cases	Deaths			Cases	Deaths	
Massachusetts:				Maryland:			
Boston	0	0	1	Baltimore	1	1	0
Ohio:				District of Columbia:			
Cincinnati	1	0	0	Washington	1	1	0
Michigan:				Texas:			
Detroit	1	0	1	San Antonio	1	0	0
Missouri:				California:			
St. Louis	1	0	0	Los Angeles	1	0	0

Encephalitis, epidemic or lethargic.—Cases: Charleston, S. C., 1.
Pellagra.—Cases: Philadelphia, 1; Atlanta, 3; Los Angeles, 1.
Typhus fever.—Cases: Charleston, S. C., 2; Houston, Texas, 2.

FOREIGN REPORTS

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—A cumulative table giving current information regarding the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS of February 23, 1940, pages 342–345. A similar table will appear in future issues of the PUBLIC HEALTH REPORTS for the last Friday of each month.

Plague

Argentina—Salta Province—El Carril.—For the period January 16 to 31, 1940, 1 case of plague with 1 death was reported in El Carril, Salta Province, Argentina.

Azores Islands—San Miguel.—During the month of January 1940, 2 cases of plague were reported in a rural area of San Miguel, Azores Islands.

Typhus Fever

France—Basses-Alpes Department—Le Caire—Correction.—The case of typhus fever reported on page 224 of the PUBLIC HEALTH REPORTS of February 2, 1940, as occurring in Le Caire, Basses-Alpes Department, France, is an error. It should have been reported as occurring in Cairo, Egypt.

Yellow Fever

Colombia—Antioquia Department—San Luis.—On January 15, 1940, 1 death from yellow fever was reported in San Luis, Antioquia Department, Colombia.

French Equatorial Africa—Gabon.—On February 13, 1940, 1 suspected case of yellow fever was reported in Gabon, French Equatorial Africa.